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Insect thermal tolerance: what is the role of ontogeny, ageing and senescence?

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ABSTRACT

Temperature has dramatic evolutionary fitness consequences and is therefore a major factor determining the geographic distribution and abundance of ectotherms. However, the role that age might have on insect thermal tolerance is often overlooked in studies of behaviour, ecology, physiology and evolutionary biology. Here, we review the evidence for ontogenetic and ageing effects on traits of high- and low-temperature tolerance in insects and show that these effects are typically pronounced for most taxa in which data are available. We therefore argue that basal thermal tolerance and acclimation responses (i.e. phenotypic plasticity) are strongly influenced by age and/or ontogeny and may confound studies of temperature responses if unaccounted for. We outline three alternative hypotheses which can be distinguished to propose why development affects thermal tolerance in insects. At present no studies have been undertaken to directly address these options. The implications of these age-related changes in thermal biology are discussed and, most significantly, suggest that the temperature tolerance of insects should be defined within the age-demographics of a particular population or species. Although we conclude that age is a source of variation that should be carefully controlled for in thermal biology, we also suggest that it can be used as a valuable tool for testing evolutionary theories of ageing and the cellular and genetic basis of thermal tolerance.

Key words: temperature tolerance, chill coma, development, ageing, knockdown, phenotypic plasticity.

CONTENTS

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I. INTRODUCTION

(1) Background

The thermal biology of ectotherms has a long and rich history in ecology and evolutionary physiology and is currently enjoying renewed attention. This is at least partly owing to the ease of temperature measurement and the rapidly growing body of molecular tools available for its investigation (Feder & Mitchell-Olds, 2003; Hoffmann, Sørensen & Loeschcke, 2003; Chown & Nicolson, 2004). Furthermore, there is increased awareness that physiological responses are a major strategy used in compensating for climate variation at several time-scales (e.g. Pörtner & Knust, 2007; reviewed in Berteaux *et al.*, 2004; Helmuth, Kingsolver & Carrington, 2005; Chown & Terblanche, 2007). The role of temperature in setting geographic distribution limits *via* its influence on population dynamics has also highlighted the need for detailed investigation of temperature biology in ectotherms (see e.g. Hargrove, 2001; McMillan *et al.*, 2005; Frazier, Huey & Berrigan, 2006; Musolin, 2007). An in-depth understanding demands an integrative approach, encompassing investigation of, for example, rate effects (e.g. Meats, 1976; Rako & Hoffmann, 2006; Terblanche *et al.*, 2007*a*), damage accumulation (e.g. Nedved, Lavy & Verhoef, 1998; Renault *et al.*, 2004; Zani *et al.*, 2005), basal and inducible temperature tolerance, temperature-sensing mechanisms and thermoregulation strategies (reviewed in Seebacher & Franklin, 2005) if ecological patterns and processes are to be thoroughly understood at broad and fine spatial scales (Chown, Gaston & Robinson, 2003; Chown & Terblanche, 2007). In addition, strong support exists for a link between thermal biology and fitness in the wild (Kristensen, Loeschcke & Hoffmann, 2007; Loeschcke & Hoffmann, 2007; Kristensen *et al.*, 2008) and, consequently, the ecological implications are profound. Finally, the role of adaptive phenotypic plasticity in thermal biology, although questioned and highly debated from a theoretical perspective (Wilson & Franklin, 2002; Woods & Harrison, 2002; Seebacher, 2005) is finding renewed empirical support (Huey *et al.*, 1999; Deere & Chown, 2006; Wilson, Hammill & Johnston, 2007; reviewed in Chown & Terblanche, 2007; and see Ghalambor *et al.*, 2007). However, the importance of intrinsic biological variation is often overlooked, or at least is not typically the focus of the investigation (Spicer & Gaston, 1999). Such variation can occur at one of several hierarchical levels each with different implications for studies of evolutionary biology (Fig. 1).

Spicer & Gaston (1999) suggest two reasons why investigation of the hierarchical nature of such physiological variation may be important. First, from a heuristic perspective, if physiological variation is a basic fact of life it would seem important to understand how this diversity is distributed. Second, an understanding of physiological diversity would aid comprehension of the circumstances in which physiological variation at levels other than that which is being explicitly studied may or may not be of major concern. In insects, few studies have attempted to document physiological variation at three or more hierarchical levels (Fig. 1, but see discussions in Chown, 2001; Hoffmann *et al.*, 2001).

At the individual whole-organism level, physiological variation associated with ontogeny has been well documented across a variety of traits (Spicer & Gaston, 1999; Chown & Nicolson, 2004). However, with the obvious exception of senescence, physiological variation within life stages has received less attention (but see e.g. Queathem &

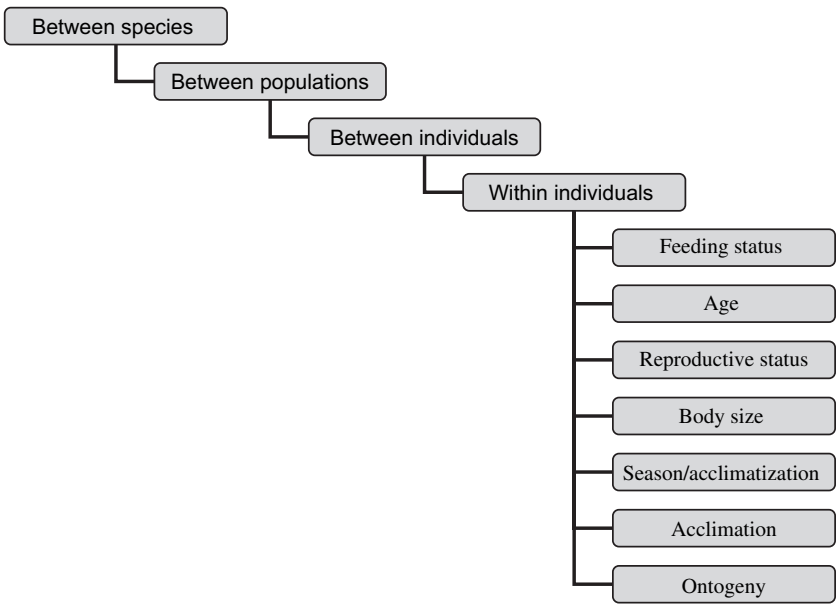

Fig. 1. Schematic diagram of the hierarchical classification for commonly accepted levels of variability in physiological traits (see Spicer & Gaston, 1999 for discussion). Included in the figure are several of the more common factors affecting within-individual variability of physiological tolerance.

Full, 1995; Gray & Bradley, 2003; Terblanche, Klok & Chown, 2004). Moreover, the degree to which factors such as age, gender, feeding and reproductive status influence the state of a physiological trait within individuals in a population has generally been poorly documented in insects (Chown & Nicolson, 2004).

Individuals can also show physiological variation in response to daily or seasonal changes in abiotic factors. This has been demonstrated in insects mainly in response to temperature (e.g. Sinclair *et al.*, 2003*b*), desiccation (e.g. Parsons, 1982; Gibbs, Chippindale & Rose, 1997; Gibbs & Matzkin, 2001) and metabolic rate (e.g. Davis *et al.*, 2000) and other performance traits (e.g. locomotion performance). Many of these changes are discussed under the rubric of hardening or acclimation (see below) and are reviewed elsewhere (eg. Spicer & Gaston, 1999; Chown & Nicolson, 2004).

Among-individual differences are generally related to differences in genotype between individuals and differences resulting from genotype-environment interactions, such as acclimation, and 'maternal' or 'parental' effects (discussed in e.g. Falconer & Mackay, 1996; Mousseau & Fox, 1998; Magiofoglou & Hoffmann, 2003). However, if age is an unknown factor this may contribute to among-individual variation in phenotypes.

(2) Review aims

The aim of this review is to draw attention to the fact that measures of thermal tolerance, and likely also the plasticity therein, are not static over the physiological age of insects and, by contrast, often vary dramatically. In some species for a particular trait of temperature tolerance this appears to follow systematic patterns of variation while in other cases the variation does not appear to hold any generalities. This has implications for research focusing on the genetic basis of temperature limits in insects (e.g. Morgan & Mackay, 2006; Norry *et al.*, 2006; Rako *et al.*, 2007), the relationship between heat shock proteins (Hsps) and thermal tolerance (e.g. Feder, Blair & Figueras, 1997; Sørensen & Loeschcke, 2002; McMillan *et al.*, 2005), and resistance acclimation and thermal resistance in natural populations and/or species (e.g. Kellett, Hoffmann & McKechnie, 2005; Terblanche *et al.*, 2007*a*; Jensen, Overgaard & Sørensen, 2007). Therefore the primary goal of this review is to draw attention to the fact that thermal resistance is a trait whose value is not fixed, but changes dramatically in an age-dependent manner. Although the acclimation and treatment conditions are usually carefully controlled in thermal tolerance experiments (e.g. Terblanche *et al.*, 2006; Kristensen *et al.*, 2008), we here draw attention to the effect of ontogeny on thermal tolerance, and in particular we wish to review the substantial literature that demonstrates ageing, ontogenetic or senescence effects on thermal biology. Furthermore, because the age-dependent variation in thermal tolerance may be classified into some basic patterns from the empirical data, a secondary goal of this review is to outline the key hypotheses which might explain the variety of patterns. Finally, rather than simply controlling for physiological age of an organism in thermal biology, we are of the opinion that studies of age-related changes in thermal tolerance can complement studies of evolutionary gerontology, or the mechanisms of ageing, and can also provide novel insights into the genetic and biochemical basis of thermal tolerance. However, while we acknowledge that an ontogenetic approach will not necessarily be feasible in all insect species, particularly those with rare, elusive or microscopic life stages, there are plenty of insect species available to address the key issues at hand.

(3) Thermal semantics

The physiological responses of insects to temperature extremes can be measured in a variety of ways. However these have been extensively reviewed previously and are therefore not covered in detail here (see e.g. Cossins & Bowler, 1987; Lutterschmidt & Hutchison, 1997*a, b*; Feder & Hofmann, 1999; Hoffmann *et al.*, 2003; Chown & Nicolson, 2004; Sinclair & Roberts, 2005 for access to this literature). Similarly, insect low temperature responses have been reviewed broadly within the context of freeze tolerance and intolerance and the various strategies insects adopt at low temperatures and we therefore do not review these topics either (reviewed in Storey & Storey, 1992; Danks, 1996; Denlinger & Lee, 1998*a*; Sinclair, Addo-Bediako & Chown, 2003*a;* Sinclair *et al.*, 2003*c*; Bale, 2002; Chown & Nicolson, 2004; Turnock & Fields, 2005; Chown & Terblanche, 2007). However, for the purposes of this review it is important to point out that responses to temperature change can differ in subtle but distinct ways and may even reflect completely different underlying processes (see in particular Hoffmann *et al.*, 1997; Berrigan, 2000; Folk *et al.*, 2006), from gene expression to biochemical regulation and through to how the response is manifested by the animal as an individual (i.e. at the level of the phenotype). Therefore, caution must be exercised when drawing comparisons between different measures of temperature tolerance [e.g. critical thermal maxima *versus* knockdown resistance (KDR) (Folk, Hoekstra & Gilchrist, 2007)].

The usage of many terms in this field of insect thermal biology is inconsistent and, in consequence, potentially confusing in the literature. This is particularly true for the following terms: acclimation, acclimatization, heat and cold shock, heat and cold stress, hardening, heat and cold resistance and heat and cold tolerance. This issue has recently been the subject of several commentaries and we direct readers to these (Bowler, 2005; Sinclair & Roberts, 2005, Loeschcke & Sørensen, 2005; Lagerspetz, 2006). In particular, these debates emphasize that the usage of these terms is a complicating feature of the literature although all induced changes within a particular trait broadly fall under the rubric 'phenotypic plasticity' (Chown & Terblanche, 2007). In consequence, the complexities in usage of these terms are briefly outlined in Table 1. In particular, readers should be aware that the methods used (e.g. acclimation, shock and hardening) to effect phenotypic changes in thermal tolerance may not be the same throughout the literature, and so the physiological and biochemical outcomes found may not offer an explanation of a mechanism(s) consistent with the treatment employed. Critically, all measures of temperature

tolerance in insects are dependent on the time at a particular treatment, relative to the magnitude of the change and the rate at which the temperature was altered (see e.g. Terblanche *et al.*, 2007*a* and references therein). Further complicating matters, how the animal responds to a particular time-temperature treatment depends, at least to some degree, on the animal's thermal history (e.g. Buckley, Owen & Hofmann, 2001). Essentially, thermal limits are highly relativistic and this should be borne in mind throughout this review.

(4) High- and low-temperature tolerance: fundamental differences

Resistance to thermal extremes varies in three principal ways between low and high temperatures in terrestrial arthropods. Firstly, the range of variation found is considerably less at high temperatures by comparison with low temperatures at the interspecific level (Gaston & Chown, 1999; Addo-Bediako, Chown & Gaston, 2000; Goto, Kitamura & Kimura, 2000; Kimura, 2004; reviewed in Chown & Nicolson, 2004). Similarly, within species there is more variation of resistance to low temperature than there is to high temperatures (Chown & Nicolson, 2004; Terblanche *et al.*, 2007*a*). For example, at the interspecific level, critical thermal maxima (*CTmax*) varied less than critical thermal minima (*CTmin*) among dung beetle species across an altitudinal gradient in South Africa (Gaston & Chown, 1999; see also Chown, 2001). Similarly, using ten *Drosophila* species, Goto *et al.* (2000) showed considerably less variation in upper lethal temperature by contrast with lower lethal temperatures (see their Fig. 4). At the intra-specific level, in the tsetse fly *Glossina pallidipes* the responses of *CTmax* to different rates of heating and starting temperatures were in the order of 5°C while *CTmin* changed by as much as 10°C across a similar range of cooling rates and starting temperatures (Terblanche *et al.*, 2007*a*). Selection experiments using *D. melanogaster* have confirmed this by demonstrating that low-temperature tolerance can be lowered to allow increased cold resistance independently of high-temperature tolerance and without trade-offs in multiple life-history traits (Anderson, Hoffmann & McKechnie, 2005; but see Kristensen *et al.*, 2008).

The reasons for these differences between high- and low-temperature thermal tolerance are unclear but may be partly due to different genetic bases for the traits (Hercus *et al.*, 2000; Anderson *et al.*, 2005; but see Norry, Gomez & Loeschcke, 2007) and/or more mechanistic facets at sub-cellular, tissue and organ, and whole-animal levels involved in low-temperature tolerance (see discussion in Klok, Sinclair & Chown, 2004). At high temperatures, it seems likely that fewer hierarchical factors within the animal contribute to thermal tolerance. For *CTmin* and *CTmax* determined under different oxygen partial pressures, Klok *et al.* (2004) argued that the higher levels of variation in *CTmin* might be a consequence of the number of physiological (hierarchical) levels involved. As such, high-temperature tolerance is more simply controlled by cellular level effects while low-temperature tolerance is influenced by a host of factors including reduced function of Na^+/K^+-ATPase pumps in critical tissues, thus the inability to generate action potentials and regulate ion balance (Anstee & Bowler, 1979; Peacock, Bowler & Anstee, 1976). In consequence, a lack of ATP in the mitochondria may be in part determined by efficacy of the oxygen delivery system and might impact on low temperature tolerance (see Hardewig, Peck & Pörtner, 1999; Sokolova & Pörtner, 2003; Klok *et al.*, 2004 and references therein). However, further work is required to address precisely why and how these differences between

Table 1. Some terminology and concepts used in studies of the thermal biology of insects. All of the treatments can induce some form of phenotypic plasticity although the time scales and underlying mechanisms which induce these responses can vary greatly (see e.g. Fig. 1 in Sinclair & Roberts, 2005; reviewed in Loeschcke & Sørensen, 2005; Bowler, 2005; Lagerspetz, 2006; Chown & Terblanche, 2007). Generally hardening, shock, acclimation and acclimatization are considered as treatments, however sometimes they are also considered as a physiological response or outcome. We have tried to indicate the most common usage of the terminology here (Treatment/outcome). A further caveat is that all traits, and thus variables measured, are virtually meaningless from a thermal biology perspective without some knowledge of both time (i.e. experimental duration) and temperature

Phenotypic plasticity	Time effect of the phenotypic change	Treatment/outcome
Hardening	Minutes-hours	T/O
Shock	Minutes-hours	T
Acclimation (laboratory conditions)	Minutes-weeks	T/O
Acclimatization (field conditions)	Days-weeks	O
General method*	**Trait (parameter/variable)**	**Example Reference**
	Tolerance	
Static	Survival/fecundity (% alive *versus* dead)	Goto *et al.* (2000)
Dynamic	Tolerance (time or temperature)	Goto *et al.* (2000)
	Resistance	
Static	Knockdown resistance (time x temperature)	Jensen *et al.* (2007)
Dynamic	Critical thermal limits (temperature)	Klok & Chown (2001)
	Recovery	
Dynamic	Chill coma recovery (time x temperature)	David *et al.* (1998)

*following methodological definitions by Lutterschmidt & Hutchison 1997*b*.

upper and lower thermal limits exist and the extent of this pattern among insect species.

Second, there is a greater magnitude of plasticity at low temperatures in response to say a similar acclimation temperature range, such that the extent of response values are greater in low-temperature tolerance estimates by contrast with high-temperature tolerance (see e.g. Goto *et al.*, 2000; Klok & Chown, 2003). Similar results have been shown in vertebrate ectotherms such as reptiles (Kingsolver & Huey, 1998). In addition, from work undertaken on marine invertebrates, Stillman (2003) has proposed that basal tolerance to heat and cold is traded off against the magnitude of the acclimation response. Whether or not this is the case in insects, either for upper or lower thermotolerance, is not well established, although recent evidence from the diving beetle genus *Deronectes* (Dytiscidae) shows that the magnitude of upper lethal temperature acclimation responses is positively related to basal upper lethal temperatures and therefore appears to counter Stillman's (2003) hypothesis (Calosi, Bilton & Spicer, 2008; but see also discussion in Chown & Nicolson, 2004; Kristensen *et al.*, 2008). However, whether this pattern holds more broadly for other insect groups remains under-explored.

Finally, intraspecific comparisons along latitudinal gradients show opposing clines for low- and high-temperature thermal tolerance in *D. melanogaster* which suggests that upper and lower temperature tolerance can vary with temperature as a function of latitude, and this variation is likely a consequence of direct or indirect effects of natural selection (Hoffmann, Anderson & Hallas, 2002; Rako *et al.*, 2007; see also Chen & Kang, 2004; Blows & Hoffmann, 2005; Hoffmann & Weeks, 2007).

II. EMPIRICAL EXAMPLES

Multiple biotic and abiotic factors have been identified as potentially influencing traits of thermal biology in insects (Table 2). Furthermore, the role of ontogeny or development as a major factor influencing physiological variation has long been recognized (reviewed in e.g. Spicer & Gaston, 1999; Chown & Nicolson, 2004). Senescence, as a form of age-related variation, can also contribute to physiological variation within populations or species. To avoid confusion we refer to ontogenetic variation to imply developmental (growth) effects, whilst the effects typically associated with a reduction in performance occurring in later life (or old age), typically some time after reproductive maturity has been achieved, are referred to as senescence. Although distinctions between the end of development and the onset of senescence are somewhat arbitrary, it is important to be aware that very few studies of thermal tolerance actually consider 'senescence effects' when referring to age-related changes in thermal tolerance (but see Bowler & Hollingsworth, 1966; Luckinbill, 1998; Scannapieco *et al.*, 2007). Indeed, the majority of age-effects reported in the insect physiology literature actually refer to variation occurring in early adult life (e.g. Terblanche *et al.*, 2004), and such variation in thermal tolerance may be more closely related to the physiological re-organisation associated with morphological transitions between life stages or moulting events than senescence or ageing *per se*. While developmental stage (e.g. instar number in hemimetabolous insects, or life stage in holometabolous insects) clearly influences thermal tolerance, and apparently even preferred body temperature in some cases (Rossolimo, 1997), the functional and mechanistic explanations for such variation are remarkably limited (see Section II. **7**).

(1) High-temperature responses

For high-temperature responses, the most commonly reported variation in thermal tolerance is that associated with different life stages (e.g. Coyne, Bundgaard & Prout, 1983; Krebs & Loeschcke, 1995*a*, *b*; Crill, Huey & Gilchrist, 1996; Gilchrist, Huey & Partridge, 1997; Klok & Chown, 2001). Age-related changes in upper thermal tolerance

Table 2. Biotic and abiotic factors potentially influencing traits of insect thermal biology

Effect/factor	Trait	Example reference
Photoperiod	Cold survival	Lanciani *et al.* (1992)
Density	High-temperature knockdown resistance	Bubli *et al.* (1998)
Time of day	Supercooling point; Hsp expression; cold survival, critical thermal minima	Sinclair *et al.* (2003*b*); McMillan *et al.* (2005); Kelty (2007)
Latitude/altitude	Upper and lower lethal limits, critical thermal limits, Preferred body temperature	Addo-Bediako *et al.* (2000); Hoffmann *et al.* (2002); Klok & Chown (2003); Samietz *et al.* (2005)
Caste	Critical thermal limits	Mitchell *et al.* (1992)
Gender	Chill coma recovery; heat-induced hormesis	David *et al.* (1998); Sørensen *et al.* (2007)
Acclimation	Critical thermal limits, chill coma recovery, heat/cold survival; bait re-capture	Meats (1976); Bubliy *et al.* (2002); Terblanche *et al.* (2005; 2006); Rako & Hoffmann (2006); Kristensen *et al.* (2008)
Acclimatization	Critical thermal limits	Terblanche *et al.* (2006)
CO_2 anaesthesia	Cold/heat survival	Yocum & Denlinger (1994); Wu *et al.* (2002); Nilson *et al.* (2006); Milton & Partridge (2007)
Parental/genetic lineage	High-temperature survival	Hoffmann *et al.* (2001); Zatsepina *et al.* (2001)
Pigmentation	Preferred body temperature	Forsman *et al.* (2002); Ahnesjö & Forsman (2006); reviewed in Clusella-Trullas *et al.* (2007)

Hsp, heat shock protein.

during a single life stage are discussed below in Section II. **3**. It is worth noting, however, that far less work has described high-temperature age-related effects relative to the number of studies undertaken at the low-temperature spectrum.

Life-stage effects have also been well documented for high-temperature tolerance, although Davison (1969) provides one of the few reports on changes in heat resistance throughout the complete life cycle of an insect (*Calliphora erythrocephala*) (Fig. 2). In the sub-Antarctic kelp fly, *Paractora dreuxi*, Klok & Chown (2001) found that field-collected larvae had a mean *CTMax* of 35.5°C as opposed to field-collected adults which had a *CTMax* of 30.2°C. Overall, larvae of *P. dreuxi* had 8.5°C greater thermotolerance range (i.e. between high and low critical thermal limits) than adults. After temperature selection for approximately 100-275 generations Gilchrist *et al.* (1997) found little response to temperature selection on high-temperature survival in eggs across replicated laboratory lines of *D. melanogaster* (i.e. all high- and low-temperature lines had similar high-temperature survival in eggs). By contrast, adult survival to high temperatures was positively related to selection temperature (i.e. high-temperature lines had higher survival), thus indicating a dramatic difference between life stages in temperature tolerance and their response to laboratory selection (see also Tucić, 1979; Krebs & Loeschcke, 1995*b*).

These results generally support the notion that high-temperature limits decline with age and into more advanced life stages (but see Krebs & Loeschcke, 1995*b* for an obvious exception), although evidence to date suggests that the form of this negative relationship can be either non-linear (Bowler, 1967) or linear (e.g. Lamb & McDonald, 1973; Pappas *et al.*, 2007) within life-stages. In addition, several studies have demonstrated major differences in terms of insect life-stage responses to temperature selection (e.g. Tucić, 1979; Krebs & Loeschcke, 1995*b*; Gilchrist *et al.*, 1997). These differences may largely be due to variation in mobility of a particular life stage, and indeed, one may expect greater thermotolerance in life stages of insects that are less mobile. Most insects become increasingly more mobile during ontogeny, for example, as they develop flight capacity and initiate foraging and mate-seeking behaviour in holometabolous insects. Thus, the general expectation may be a decline in thermotolerance coupled with increasing behavioural compensation of temperature extremes. To our knowledge, however, no studies have explicitly attempted to dissect mobility from ontogenetic variation in thermotolerance (though see Chown & Terblanche, 2007; Terblanche, Marais & Chown, 2007*b* and discussions therein). Future studies may gain insight into such variation of thermal tolerance through e.g. genetic interference/manipulation.

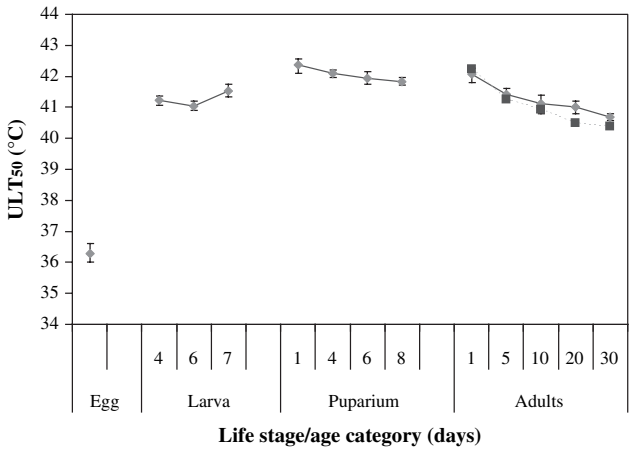

Fig. 2. Changes in temperature tolerance with age in *Calliphora erythrocephala* from Davison (1969) (for eggs, the % hatching was used; for larvae and pupae, the % success to eclosion was used; for adults, % survival was used). Values are means ± S.D. For adults, males are square symbols and females are diamond symbols. ULT_{50} is the temperature at which 50% population mortality is estimated. For further details see Davison (1969). [Re-drawn from Davison (1969) with permission from Elsevier.]

We are of the opinion that the issue may be more complex since one would thus specifically expect eggs always to be more tolerant than wandering or mobile life stages. Yet consideration of high-temperature tolerance data for *Calliphora erythrocephala* (Fig. 2) clearly shows that eggs are the least tolerant despite being a highly immobile life stage. Similarly, at low temperatures, the least mobile stage is not always the most tolerant (Section II. **2**). It seems more reasonable, therefore, that several potentially interacting factors ultimately set thermal tolerance in a given life stage and may involve trade-offs. These trade-offs probably include, but are not necessarily limited to, typical microclimate conditions experienced by the life stage, the degree of behavioural compensation that can be employed under these conditions, and the costs of compensating either behaviourally or physiologically (e.g. acclimatizing) (see, for example, discussion regarding costs and benefits of thermoregulation in Huey & Slatkin, 1976; Huey, Hertz & Sinervo, 2003; and see Chown & Terblanche, 2007). Clearly the issue is more complex than previously suspected and requires further experimental investigation.

(2) Low-temperature responses

Probably the most frequently documented variation in low-temperature thermotolerance of insects associated with ontogeny is that attributed to variation among life stages (e.g. Tucić, 1979; Chen, Denlinger & Lee, 1987; Punzo & Huff, 1988; Bale, 1991; Johnston & Lee, 1990; Czajka & Lee, 1990; Brokerhof, Morton & Banks, 1993; Watson & Hoffmann, 1995; Gilchrist *et al.*, 1997; Vernon & Vannier, 1996; Klok & Chown, 2001; Carrillo *et al.*, 2005; Jensen *et al.*, 2007; Terblanche *et al.*, 2007*b*). For example, *Paractora dreuxi* larvae had lower *CTmin* than adults (-5.1°C vs. -2.7°C) (Klok & Chown, 2001), while supercooling points (SCP or crystallization temperature) varied among -22.6, -13.0, -16.2, -16.9, -18.9 and -18.7°C in eggs, second instar larvae, final-instar larvae, pupae, adult females and adult males, respectively, of the tineid moth *Tineola bisselliella* (Chauvin & Vannier, 1997). Notably, Carrillo *et al.* (2005) found a general decline in SCP values over several

developmental stages in the chill-susceptible Indianmeal moth *Plodia interpunctella*, suggesting improved low-temperature tolerance with age (see also Carrillo & Cannon, 2005). However, a decline in low-temperature tolerance with ontogeny cannot be considered universal since some studies have shown a lack of directional change in SCPs (e.g. Tucić, 1979) and other traits (e.g. Pitts & Wall, 2006; Cho *et al.*, 2007).

Since so many studies reported SCP variation among insect life stages, we were able to compile a database for several Diptera species. From this synthesis, it is readily demonstrated that SCPs vary greatly during ontogeny within various insect species (Fig. 3), and this is not necessarily a simple, progressive (linear) increase in SCP, as one might expect if mobility was a key determining factor. At low temperatures, Chen *et al.* (1987) showed that pupae of the fleshfly *Sarcophaga crassipalpis* were most tolerant of cold shock while adults and larvae were less tolerant. Constantinou & Cloudsley-Thompson (1986) reported a marked reduction in low-temperature freezing (i.e. higher SCP values) over development from pupae to adults in the freeze-avoiding *Tenebrio molitor*. Yet probably the most rigorous examination of between life-stage ontogenetic effects on low-temperature tolerance is a recent study undertaken by Jensen *et al.* (2007). Here, using *D. melanogaster*, Jensen *et al.* (2007) found approximately 10-15°C variation for the temperature at which 50% population mortality (LT_{50}) occurs among eggs, larvae, pupae and adults, and several age categories within each life stage. Generally eggs were the most low-temperature tolerant group, while adult females (48 h post-eclosion) were the least low-temperature tolerant (see Fig. 4).

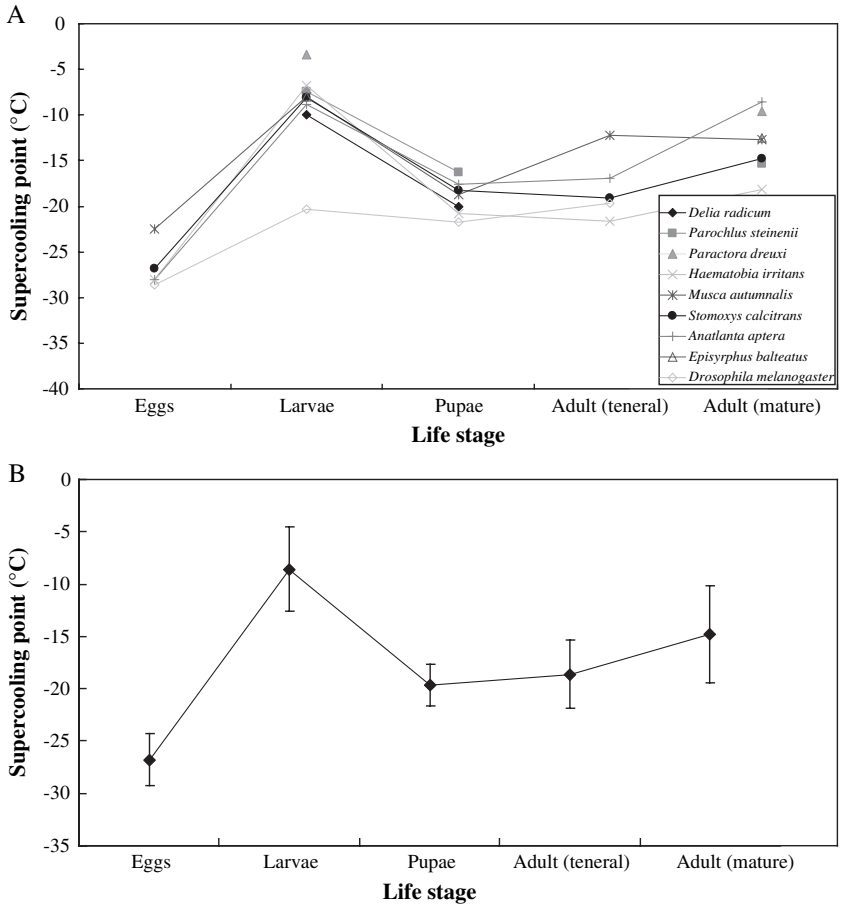

Fig. 3. Supercooling point (SCP) variation among various life stages for dipteran species extracted from the published literature. We used the following selection criteria for a study's inclusion: first, to avoid undue methodological variation species were included only if two or more life stages had been reported within the same study. In cases where the same species was presented in several studies we gave precedence to values from the study with the most life stages reported, then the study with the cooling rate nearest to 0.1°C/min, then the study presenting data with animals held for the least amount of time in the laboratory and finally, if all else was equal, we chose the most recent study. Gender effects were disregarded and sexes pooled if necessary. In cases where pre- and post-diapause samples were taken we only used the pre-diapause data. (A) Raw data for each species with multiple life-stage SCP reported. (B) Mean ($\pm$1 S.D.) SCP calculated across species for each life stage. Sources: *Delia radicum*: Kostal (1993); *Parochlus steinenii*: Shimada *et al.* (1991); *Paractora dreuxi*: Klok & Chown (2001); *Haematobia irritans*: Jones & Kunz (1997); *Musca autumnalis*: Rosales *et al.* (1994); *Stomoxys calcitrans*: Jones & Kunz (1997); *Anatlanta aptera*: Vernon & Vannier (1996); *Episyrphus balteatus*: Hart & Bale (1997); *Drosophila melanogaster*: Tucić (1979).

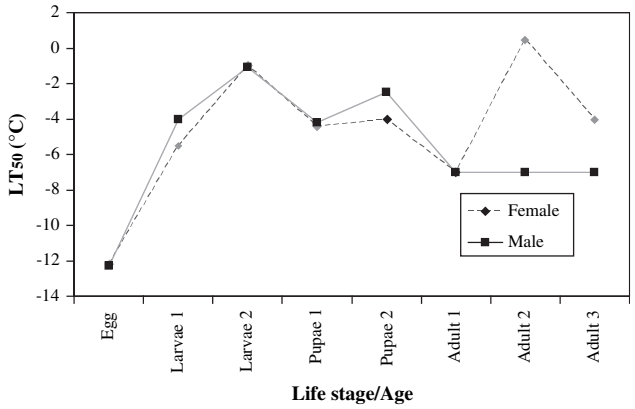

Fig. 4. Effect of ontogeny and life stage on the lethal temperature (LT_{50}) of *Drosophila melanogaster* exposed to cold shock for 1 h. Full explanation of life stages and confidence limits are given in Jensen *et al.* (2007). [Re-drawn from Jensen *et al.* (2007) with permission from Elsevier.]

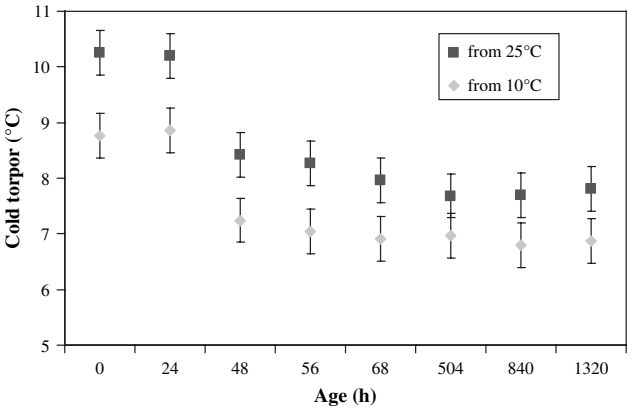

Fig. 5. Variation in mean (±1 S.E.M.) low-temperature torpor (in °C) with adult age in the Queensland fruit fly *Dacus tryoni*. Two groups of flies are shown, those reared at 25°C and at 10°C. [Data re-drawn from Meats (1973) with permission from Elsevier.]

It is clear, however, that within-life-stage effects also contribute to low-temperature thermotolerance variation (e.g. Burnett, 1957; Tucić, 1979; Burks, Hagstrum & Baker, 1999; Zhao, Hao & Kang, 2005; Wang & Kang, 2005; Jensen *et al.*, 2007). Indeed, a wide variety of low-temperature tolerance traits have been reported to vary within life stages in insects. Early research by Meats (1973) identified a significant reduction in low-temperature torpor in the fruitfly *Dacus tryoni* over the first 1-2 days of adult life. Thereafter, from days 3 to 56 torpor temperatures remained fairly constant (Fig. 5). In addition, David *et al.* (1998) reported that within adult *D. melanogaster* chill coma recovery times increased among 3 to 29 day old flies. Again, it's difficult to generalize these results across species since some exceptions do occur. For example, Carrillo, Cannon & Ferrington (2004) reported no effect of time spent in the laboratory for adults of the chironomid *Diamesa mendotae* suggesting limited within-life-stage ageing effects, while in young (Stage I) *D. melanogaster* larvae SCPs were approximately 6°C lower than in older (Stage II) larvae irrespective of whether or not they had been selected for low-temperature survival for 47 generations (Tucić, 1979).

(3) Age-dependent changes in thermal tolerance in the young adult

The first record of an age-dependent effect on adult thermal tolerance was probably by Baldwin (1954) on a parasitic chalcid *Dahlbominus fuscipennis*. He reported a dramatic decrease in thermal tolerance during the first 96 h of adult life. Several demonstrations from a variety of genera confirm that the thermal resistance of some holometabolous insects is not constant during adult life. Typically, it is highest at eclosion and then declines during the early part of adult life to a stable lower level, and therefore is not a senescence (ageing) phenomenon *per se* (see Bowler & Hollingsworth, 1965; Bowler, 1967). In a more detailed study on the blowfly *Calliphora erythrocephala*, Davison (1969) suggested that the decline in tolerance in the early adult was the loss of high-temperature resistance required by the immobile pupal stadium and so may be a developmental 'carry-over' effect. Service *et al.* (1987) in *D. melanogaster* and Dahlgaard, Krebs & Loeschcke (1995) in *Drosophila buzzatii*, have also found this effect but in both cases it was interpreted as senescence. More recently this has also been demonstrated in *D. melanogaster*, particularly with reference to a relationship with changes in the expression of Hsps with high-temperature hardening and a loss of high-temperature resistance (Feder *et al.*, 1997, Sørensen & Loeschcke, 2002).

Davison (1969, 1971) probably offered one of the fullest demonstrations of the age-dependent decline in heat resistance in an insect and for convenience these data are presented in Tables 3 and 4. Two principal points emerged from these data. First, as can be seen in Table 3, flies reared and maintained at 24°C show a dramatic decline in tolerance to 41.2°C in the first 10 days of adult life ($p < 0.001$), and thereafter, up to 30 days their thermal resistance was unchanged. The second point is that this decline in resistance in the early adult is itself temperature dependent. In flies transferred to lower temperatures at eclosion the decline in resistance was slower, for example, when transferred to 15°C flies were more heat resistant after 10 and 15 days than similar flies maintained at 24°C, and in flies transferred to 5 or 10°C their heat resistance remained higher after 20 days as compared with flies maintained at 24°C. Note that these flies all had an identical developmental background to eclosion. It is only after eclosion that their temperature experience differed. However, when transferred to temperatures higher than 24°C at eclosion a more complex relationship was found. At 29°C the decline was somewhat faster particularly after 5 days as compared with flies at 24°C, but thereafter the levels of resistance were not different up to 30 days. When transferred to temperatures of 30°C and higher a progressive effect of warm acclimation can be seen, this is most

Table 3. Acclimation-temperature dependence of age-related changes in heat survival (LD_{50}, mean ± S.E.M., in min) at 41.2°C for male adult blowflies *Calliphora erythrocephala* developmentally acclimated to 24°C. Sample size is shown in parentheses. Blowflies were reared at 24°C to eclosion and on the day of eclosion the newly emerged adults were transferred to one of the stated temperatures between 5 and 34°C and maintained there. The thermal tolerance of these flies was determined at the stated ages. Data are from Davison (1971). Effects of acclimation temperature and age are generally significant ($p < 0.05$), but readers should see Davison (1971) for complete statistical analyses and results

Temperature (°C)	Days of age						
	1	3	5	10	15	20	30
5			95.0 ± 2.28 (18)	97.3 ± 5 (30)		84.4 ± 6.4 (18)	85.8 ± 5.74 (20)
10			86.9 ± 6.6 (18)	69.8 ± 6.4 (18)		68.7 ± 4.5 (20)	42.5 ± 3.1 (18)
15		83.8 ± 5.9 (20)	80.6 ± 3.4 (20)	49.7 ± 4.4 (18)	52.3 ± 4.3 (20)	48.3 ± 2.5 (38)	42.5 ± 2.0 (31)
19		92.2 ± 4.7 (29)	76.5 ± 5.7 (20)	43.2 ± 2.9 (36)	39.3 ± 2.2 (37)	43.1 ± 3.5 (18)	39.8 ± 4.2 (13)
24	97.4 ± 2.7(52)	87.1 ± 4.1 (18)	72.3 ± 5.1 (20)	37.2 ± 2.9 (18)	39.3 ± 2.2 (38)	39.8 ± 2.8 (20)	38.8 ± 2.7 (20)
29		85.3 ± 6.8 (18)	52.9 ± 6.0 (33)	44.7 ± 3.5 (18)	48.0 ± 2.7 (18)	43.3 ± 2.9 (18)	34.7 ± 3.0 (29)
30			78.3 ± 7.4 (18)	45.0 ± 3.1 (16)			
31			76.9 ± 6.5 (17)	54.0 ± 5.3 (20)			
32			108.5 ± 6.9 (20)	94.0 ± 8.2 (17)			
34		104.1 ± 8.7 (20)	117.2 ± 2.1 (30)	86.4 ± 7.7 (18)	79.0 ± 6.2 (20)	71.0 ± 6.8 (20)	64.4 ± 6.4 (8)

obvious at 32 and 34°C. However, the attainment of higher levels of resistance at 34°C is greatest after 5 days, but thereafter declines progressively even up to 30 days.

These data on the temperature dependence of the decline in thermal resistance with adult age show that the effect of thermal acclimation on heat resistance is complex, and compromised by developmental changes in resistance. There are reports in the literature where similar paradoxical acclimation occurs. For example, Terblanche *et al.* (2005) found the upper critical thermal limits (*CTmax*) to be higher in beetles acclimated at 12°C than at 19°C this could be explained by temperature-dependent developmental changes in heat resistance similar to those discussed above.

Another early study investigating the effect of irradiation on the ageing process reported age-related changes in heat tolerance of *D. melanogaster*. Lamb & McDonald (1973) clearly showed a negative linear relationship between survival time at 35°C and age across 3 - 43 day-old irradiated and control flies (Fig. 6). Here, a remarkable 81% of the variation in high-temperature tolerance could be explained by age of the adult. While it is tempting to attribute such variation to other correlated factors, such as changes in body size, this seems to be an unrealistic generalization since many adult holometabolous insects do not change body mass substantially after flight muscle development is complete (i.e. post-teneral). Change in adult size therefore is unlikely to explain the dramatic variation in Lamb & McDonald's (1973) data. More recently, similar negative linear effects have been documented in *D. melanogaster* for high-temperature knockdown resistance (KDR) (Pappas *et al.*, 2007). Here, KDR decreased with adult age from 0 to 12 days in *Drosophila melanogaster* in both sexes, whether they had been selected for KDR or not. A marked reduction in Hsp70 expression accompanied the age-related reduction in high-temperature resistance in this study, and was most evident between eclosion and 3 days of age (Pappas *et al.*, 2007; see also Sørensen & Loeschcke, 2002). By contrast, in *G. pallidipes* adult age appears unrelated to critical thermal maxima across 1 - 24-day-old flies (Terblanche *et al.*, 2006; Terblanche *et al.*, 2007*a*). Therefore, we can identify three functional relationships of high-temperature tolerance and age in adult holometabolous insects: (i) negative linear,

Table 4. Acclimation-temperature dependence of age-related changes in heat survival (LD_{50}, mean ± S.E.M., in min) at 41.2°C for male adult blowflies *Calliphora erythrocephala* developmentally acclimated to 15°C. Sample size is shown in parentheses. Blowflies were reared at 15°C to eclosion, on the day of eclosion the newly emerged adults were transferred to one of the stated temperatures between 15 and 34°C and maintained there. The thermal tolerance of these flies was determined at the stated ages. Data are from Davison (1971). Effects of age and acclimation temperature are generally significant ($p < 0.05$) but readers should see Davison (1971) for complete results

Temperature (°C)	Days of age			
	1	5	10	20
15	104.4 ± 7.0 (18)	73.0 ± 5.7 (20)	38.0 ± 2.9 (20)	39.8 ± 2.6 (20)
24		67.5 ± 5.1 (18)	33.3 ± 2.6 (18)	39.4 ± 3.1 (18)
29		40.9 ± 3.3 (18)	45.6 ± 2.8 (18)	38.3 ± 2.9 (18)
34		98.8 ± 6.2 (20)	91.0 ± 7.1 (20)	65.0 ± 5.5 (20)

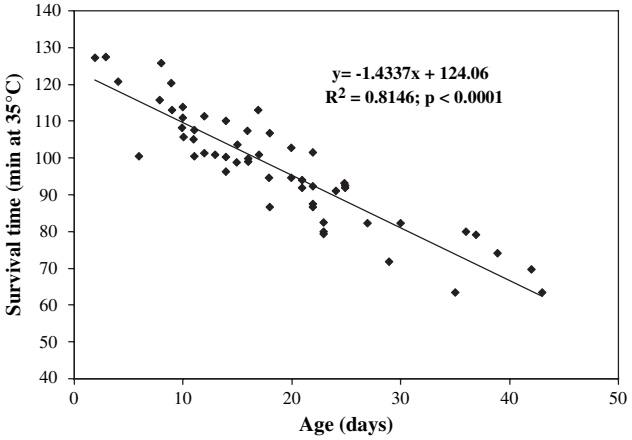

Fig. 6. Variation in high-temperature survival time with age in *Drosophila melanogaster*. The data show a linear decline [control flies only; irradiated flies which were originally presented in Lamb & McDonald (1973) were excluded from this graph]. Note that the linear regression was not fitted in the original work. [Redrawn from Lamb & McDonald (1973) with permission from Elsevier.]

(ii) negative non-linear and (iii) constant or no relationship. As yet, and importantly for theories of senescence and stress resistance (see Section **(5)** below), there is little evidence for an improvement in thermal tolerance with age, at least for high temperature survival.

(4) Does rearing temperature subsequently affect adult temperature tolerance?

Maynard Smith (1957) proposed there maybe two types of thermal acclimation in adult insects. First, a physiological acclimation that is attained in the adult stage as a result of the temperature regime experienced as an adult. This form of acclimation is usually recognized as transitory and reversible, lasting as long as the new thermal conditions persist (Bowler, 2005; Chown & Terblanche, 2007). There are many demonstrations that acclimation can alter both heat resistance and cold resistance in a variety of insects (see reviews in Denlinger & Lee, 1998*a*, *b*; Chown & Nicolson, 2004; Chown & Terblanche, 2007). Secondly, a developmental acclimation that is dependent on the temperature at which preadult stages were maintained. Maynard Smith (1957) suggested that the latter could be carried over into the adult and so was developmentally fixed (i.e. canalized).

Table 4 (from Davison, 1971) shows data for *C. erythrocephala* that had been reared to eclosion at 15°C; these data can be compared with those presented in Table 3 to assess the significance of developmental acclimation in this species. Flies reared at 15°C were transferred at eclosion to either 15°, 24°, 29° or 34°C and their survival at 41.2°C was determined after 5, 10 and 20 days at the new temperature. If developmental acclimation had occurred then flies reared at 15°C should be less resistant for each acclimation temperature and for each time point compared with flies reared at 24°C (see Table 3). The differences were not statistically significant so no support for developmental acclimation was found in this species. However, the same age and temperature-dependent decline in heat resistance occurred and as in 24°C flies warm acclimation was attained after being kept at 34°C for 10 and 20 days. Terblanche & Chown (2006) presented a similar study on the tsetse fly *Glossina pallidipes* and found a developmental acclimation effect on cold resistance but not on heat resistance. They also described a small effect of acclimation in adults in altering heat resistance but a larger and more significant effect on cold resistance (see also Terblanche *et al.*, 2006). Thus, little evidence is available to support a role for developmental acclimation in setting heat tolerance in insects (Terblanche & Chown, 2006), but there is evidence to show that physiological acclimation is an important factor in the thermal tolerance of adult insects (Maynard Smith, 1957; Levins, 1969; Nuttall, 1970; Meats, 1976; Bowler & Hollingsworth, 1965, 1966; Sørensen & Loeschcke, 2002; Klok & Chown, 2003; Terblanche *et al.*, 2005; see also extensive review by Hoffmann *et al.*, 2003). Regardless, Krebs & Loeschcke (1995*a*) have reported that 'conditioning' *Drosophila buzzatii* pupae at 38°C did cause a carryover of increased resistance in the adult, presumably owing to the Hsps produced persisting after removal from the high-temperature treatment. What was also noticeable in Terblanche & Chown (2006) was that the developmental and adult acclimation treatments did not significantly afford a combined effect [see also Zeilstra & Fischer (2005) for low-temperature responses]. Recently, Kristensen *et al.* (2008) also found large benefits of developmental cold acclimation in field release-recapture trials using *D. melanogaster*. Their results suggest that while developmental cold acclimation is more beneficial than adult acclimation it may also be more costly in terms of high-temperature performance. With respect to heat resistance in *G. pallidipes*, there was considerable unexplained variance in the data, and it was just this finding in earlier work (Bowler & Hollingsworth, 1965) that resulted in the realization that adult age had not been a controlled variable in their experimental design.

(5) Senescence and thermal tolerance

The experimental gerontology literature predicts a decline in stress resistance with senescence although the generality of this prediction has been seldom explored in the context of thermal biology (although see Rose, 1984; Service *et al.*, 1987; Minois, 2001 for access to this literature; see also reviews of ageing mechanisms by Rose, 1994; Tower, 1996; Partridge & Gems, 2002, 2006). This expectation is supported by the fact that selection for longevity improves low-temperature tolerance in *Drosophila melanogaster* (Luckinbill, 1998). Similarly, at high temperatures, there is a positive correlation between KDR and longevity within several *Drosophila* species (Scannapieco *et al.*, 2007; see also Lithgow *et al.*, 1995; Hercus, Loeschcke & Rattan, 2003; Norry *et al.*, 2006). Furthermore, Lithgow *et al.* (1995) report that a single-gene mutation in *C. elegans* confers not only an increase in longevity, but significantly, also an increase in intrinsic thermotolerance (thermal resistance). Overexpression of Hsp70 suppresses early-life egg-hatching, indicating antagonistic pleiotropy and that this trade-off contributes to

the evolution of senescence in *Drosophila melanogaster* (Silbermann & Tatar, 2000). So how much variation of thermal tolerance is associated with ontogeny or senescence, and is this sufficient to be of concern to the interpretation of ecological and evolutionary physiology research? Here, from the above review of the available literature, we argue that age-related changes are substantial and sufficiently widespread for concern as they could strongly influence the outcomes of experimental research, and hence, conclusions of evolutionary variation by natural selection.

(6) The role of heat shock proteins (Hsps)

A special role for the expression of Hsps as a determinant of both heat and cold resistance has been implied in this review, however, it is not completely demonstrated. The idea is also prompted by Bettencourt, Feder & Cavicchi (1999) who showed a correlation between ancestral rearing temperature and Hsp70 expression in *D. melanogaster*, emphasising that environmental temperature is also an important selective agent in microevolution. It is generally accepted that it is largely the ability of proteins to resist thermal perturbation that sets the upper thermal limits for a species (Somero, 2003; though see also Pörtner, 2001; Pörtner & Knust, 2007; and discussions in Chown & Nicolson, 2004; Chown & Terblanche, 2007; Lighton, 2007 for insects) and so determines basal heat tolerance. However, what is not clear is whether the inherent levels of Hsps produced in cells contribute to this basal thermal tolerance. This is a question that arises from several points of consideration above. It is clearly demonstrated that Hsp expression induced by exposure to heat and cold stress is coupled to an increase in resistance to extreme heat or cold (Yocum, Joplin & Denlinger, 1991; Feder & Hofmann, 1999; Sejerkilde, Sørensen & Loeschcke, 2003; Rinehart *et al.*, 2006). This relationship is now well established with regard to heat stress but is less well understood in relation to cold stress (Sinclair & Roberts, 2005). Furthermore, Hsp expression alone cannot account for the entirety of the increased tolerance induced by the stress (Feder & Hofmann, 1999).

It is necessary to remember that members of several families of Hsps, that differ in molecular size (Hsp10s, Hsp20s, Hsp60s, Hsp70s, Hsp90s and Hsp100s), can be expressed in the cells of an insect. These different families may have different sub-cellular, cell and tissue distributions. Some members of these families are not inducible by stress, others are expressed and have that expression up-regulated by stress, whilst others are only induced following the application of a stress (Feder, 1987; Hightower, 1991). As stated above, the stress-induced up-regulation of Hsps (in insects, studies have mostly focused on Hsp70) correlates with an increase in tolerance to applied stresses. This was elegantly demonstrated by Welte *et al.* (1993) with *D. melanogaster* larvae genetically engineered to possess additional copies of Hsp70 genes. These larvae expressed higher levels of Hsp70 following heat stress and showed increased survival at a lethal temperature. Thus, whether the levels of Hsps expressed (so-called constitutive Hsps) influence, or even determine, the basal level of heat and cold tolerance, becomes an obvious question.

There is some evidence that this is the case at high temperatures. Gehring & Wehner (1995) have shown that the ants *Cataglyphis bicolor* and *C. bombycina* can forage with body temperatures of 50°C, but accumulate Hsps (Hsp70 and 72) at lower body temperatures, Hsps that are normally only expressed following heat shock (e.g. in *Drosophila melanogaster*). Thus, this high level of expression may be a preadaptation to the shift in body temperature experienced by these ants between nest (*c.* 30°C) and foraging temperature (*c.* 50°C). At low temperatures Rinehart *et al.* (2006) showed that the larvae of the polar midge *Belgica antarctica* constitutively up-regulated members of several Hsp families (Hsps70, 90 and small Hsps) and so maintained a high inherent tolerance to low temperatures. It is significant that the application of additional thermal stress did not further enhance the expression of these Hsps nor cause induced thermotolerance. By contrast, in adults there was no constitutive up-regulation of these Hsps, but Hsp expression was induced by thermal stress which resulted in enhanced thermotolerance. Whilst there is therefore some evidence that favours an involvement of constitutively expressed Hsps in setting basal thermal tolerance, a more systematic set of experiments on a variety of different insect species is necessary to determine this relationship.

(7) Potential mechanisms of age-related variation

Generally, reports of age-dependent variation of thermal tolerance had been attributed to morphological reorganisation (e.g. Bowler, 1967) but precisely how and why this dramatically influences insect thermal tolerance is unclear. It is possible that some insects are more susceptible to temperature stress during moulting as behavioural thermoregulation could be compromised to some degree during such events. Elevated temperature tolerance during a transition phase could thus serve as an evolutionarily adaptive strategy for coping with concomitant elevated temperature risk. On the other hand, variation in thermal tolerance could simply be a by-product of morphological (i.e. cellular and tissue-level) re-organisation with little or no evolutionary significance. Finally, a change in physiological performance after transition to a new life stage could represent a 'carry-over' of the temperature tolerance from a previous life stage or age-class, and may be a feature of all holometabolous insects (Bowler, 1967). To our knowledge, however, no studies have specifically attempted to dissect these main possibilities. As such, these three alternatives represent mutually exclusive competing hypotheses regarding the function of ontogenetic variation in thermal tolerance. They can be defined as: (1) the non-adaptive morphological reorganisation hypothesis, (2) the adaptive morphological reorganisation hypothesis, and finally (3) the developmental carry-over hypothesis, respectively. Tests of these hypotheses within an inference framework (see e.g. Huey & Berrigan, 1996; Huey *et al.*, 1999; Deere & Chown, 2006) represent an important future goal for understanding

ontogenetic, age-related variation in thermal tolerance of hemi- and holometabolous insects.

III. CONCLUSIONS

(1) The overwhelming majority of evidence presented in this review suggests that the thermal tolerance of a species is highly variable and largely depends on the age of the organism. Indeed, this pattern may hold more broadly for both terrestrial and marine ectotherms (e.g. Magnusson, Crowder & Medvick, 1979; Van Damme, Bauwens & Verheyen, 1986; Winne & Keck, 2005 and references therein; but see also Paulissen, 1988).

(2) It follows that temperature tolerance can only be representative of a particular species within the context of the age-structure (demographics) in a specific environment. The implication for biogeography is that one or many life stages, perhaps the most temperature sensitive, may set the geographic distribution limit for a species. This review therefore highlights the increasing demand to understand the relationship between thermal biology and population dynamics.

(3) An immediately apparent implication for evolutionary biology is that different life stages or age groups may be selected for tolerance to temperature with differing intensity (Tucić, 1979; Gilchrist *et al.*, 1997). However, without thorough knowledge of the mechanisms underlying age-related variation the reasons for, and importance of, such variation are not clear.

(4) Addressing the relatively simple hypotheses outlined in Section II. **7** as explanations for these age-related changes, namely non-adaptive morphological reorganisation, adaptive morphological reorganisation, and developmental carry-over, represents an important future research goal.

(5) Knowledge of the mechanisms involved may provide insight into the primary forms of the relationships of temperature tolerance with adult age.

(6) More research is required examining age-related changes in thermal tolerance within a phylogenetic, inference-based context and accounting for variation in body size and the ability to compensate behaviourally. Investigation of age-related changes in the magnitude of phenotypic plasticity of temperature tolerance is also required. Some recent work has begun to explore this (e.g. Krebs & Loeschcke, 1995*b*; Jensen *et al.*, 2007; Terblanche *et al.*, 2007*b*).

(7) We are of the opinion that without determining the intraspecific factors influencing thermal tolerance, the role of temperature in determining mortality, and hence population dynamics and biogeography, will probably remain elusive.

(8) Age-related effects on the thermal tolerance of insects should not simply be controlled for or eliminated as a troublesome source of variation, but rather can be exploited to probe current knowledge of mechanisms and patterns of thermal tolerance. Indeed, age-related changes in thermal tolerance may be a powerful tool for dissecting out causal factors in physiology since temperature can be held constant under laboratory conditions whereas the organism's innate thermal tolerance changes [e.g. Smith & Yaffe, 1991; and see Roberts & Elekonich (2005) for similar discussion]. Furthermore, it may represent a unique experimental system for addressing evolutionary theories of ageing and stress resistance (Partridge & Gems, 2006). With the synthetic framework provided by this review now in position, it is more feasible to begin addressing the key issues potentially linking thermal tolerance and age in insect physiology.

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